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Applicants: Michael Hensel, David William Holden, and Jacqueline Elizabeth Shea

Serial No.: 10/763,883 Art Unit: 1645

Filed: January 23, 2004 Examiner: Khatol S. Shahnan Shah

For: *ATTENUATED SALMONELLA SP12 MUTANTS AS ANTIGEN CARRIERS*

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I the undersigned, Darren R. Ritsick, Ph.D., do hereby declare and state that:

1. I am a Scientific Advisor for Pabst Patent Group LLP.

2. On or about December 21, 2007 I contacted Charles J. Greenberg, Coordinator, Curriculum and Research Support at Cushing/Whitney Medical Library at Yale University via e-mail to inquire when the library received a copy of Deiwick et al., Journal of Bacteriology, 180(18):4775-4780. A copy of the e-mail string is attached as Exhibit A.

3. On December 21, 2007, Mr. Greenberg responded to my inquiry indicating that bound print volume I identified in my earlier e-mail was stamped as checked in and received by the Cushing/Whitney Medical Library on September 21, 1998.

4. On December 21, 2007, I also contacted Mr. Christopher Henry of the Welch Medical Library at Johns Hopkins Medical Institutions via telephone to inquire when the library received a copy of Deiwick et al., Journal of Bacteriology, 180(18):4775-4780.

5. On December 21, 2007, Mr. Henry responded to my inquiry via e-mail enclosing a copy of the date-stamped volume of the Journal of Bacteriology containing Deiwick et al., Journal of Bacteriology, 180(18):4775-4780. The volume was date-stamped September 14, 1998. A copy of this e-mail and a copy of the date-stamped volume are attached as Exhibits B and C.

4. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

December 27, 2007
Date

Darren R. Ritsick, Ph.D.

Charlie Vorndran

From: Darren Ritsick
Sent: Sunday, December 23, 2007 3:22 PM
To: Charlie Vorndran
Subject: Fw: Journal of Bacteriology

Sent from my Verizon Wireless BlackBerry

-----Original Message-----
From: "Charles Greenberg" <charles.greenberg@yale.edu>

Date: Fri, 21 Dec 2007 17:17:27
To:"Darren Ritsick" <darren@pabstpatent.com>
Subject: Re: Journal of Bacteriology

Hi, Dr. Ritsick-

The bound print volume you identified with the article on pages 4775-4780 was stamped as checked in and received by our library on September 21st, 1998. It would normally make it to the shelf within 24-48 hours.

If you need a copy of the page with the date stamp, please request the cover page of that volume and issue using the form: http://www.med.yale.edu/library/docdelivery/ddmacsjn.php

Best regards,
Charles J Greenberg

Quoting Darren Ritsick <darren@pabstpatent.com>:

> Dear Mr. Greenberg:
>
> Can you please provide me with the date that the following journal
> article was received by the Harvey Cushing/John Hay Whitney Medical
> Library at Yale University?
>
> Deiwick, et al., Journal of Bacteriology, Vol. 180, No. 18, pp.
> 4775-4780
>
> Thank you,
>
> Darren Ritsick, Ph.D.
>
> Scientific Advisor
> Pabst Patent Group LLP
> 400 Colony Square, Suite 1200
> 1201 Peachtree Street

Exhibit A
USSN 10/763,883

> Atlanta, GA 30361
>
> E-mail: darren@pabstpatent.com <mailto:darren@pabstpatent.com>
>
> Telephone: 404-879-2168
>
> Facsimile: 404-879-2160
>
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Charles J. Greenberg charles.greenberg@yale.edu
Coordinator, Curriculum and Research Support Tel: 203-737-2960
Cushing/Whitney Medical Library FAX: 203-785-4369
Yale University http://info.med.yale.edu/library/

Charlie Vorndran

From: Darren Ritsick
Sent: Sunday, December 23, 2007 3:21 PM
To: Charlie Vorndran
Subject: Fw: Copy of cover of Journal of Bacteriology

Ritsick.pdf (72 KB)

Sent from my Verizon Wireless BlackBerry

-----Original Message-----
From: "CHRISTOPHER HENRY" <ceh@jhmi.edu>

Date: Sun, 23 Dec 2007 14:15:06
To:<darren@pabstpatent.com>
Subject: Copy of cover of Journal of Bacteriology

Hello Mr. Ritsick,

Here is the cover of the journal you inquired about.
I hope this is satisfactory.

Thank you and Have a Merry Christmas.

Christopher Henry
Welch Services Center staff
Welch Medical Library
Johns Hopkins Medical Institutions

Exhibit B
USSN 10/763,883

JOURNAL OF BACTERIOLOGY

ASM

Salmonella typhimurium DT104: A virulent and drug-resistant pathogen

Cornelius Poppe, Nonie Smart, Rasik Khakhria, Wendy Johnson, John Spika, John Prescott

Abstract — *Salmonella typhimurium* phage type (PT) or definitive type (DT) 104 is a virulent pathogen for humans and animals, particularly cattle. It has been isolated increasingly from humans and animals in the United Kingdom and several other European countries and, more recently, in the United States and Canada. Humans may acquire the infection from foods of animal origin contaminated with the infective organism. Farm families are particularly at risk of acquiring the infection by contact with infected animals or by drinking unpasteurized milk. The symptoms in cattle are watery to bloody diarrhea, a drop in milk production, pyrexia, anorexia, dehydration and depression. Infection may result in septicemic salmonellosis and, upon necropsy, a fibrinonecrotic enterocolitis may be observed. The infection occurs more commonly in the calving season than at other times. Feedlot cattle and pigs may also be affected. Prolonged carriage and shedding of the pathogen may occur. Symptoms in humans consist of diarrhea, fever, headache, nausea, abdominal pain, vomiting, and, less frequently, blood in the stool. *Salmonella typhimurium* DT104 strains are commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline.

Résumé — ***Salmonella typhimurium* DT104 : un pathogène virulent et résistant aux médicaments.** *Salmonella typhimurium* type phagique (TP) ou type définitif (DT) 104 est un pathogène virulent pour l'homme et les animaux, particulièrement les bovins. Il a été de plus en plus souvent isolé chez les humains et chez les animaux au Royaume-Uni et dans plusieurs autres pays européens et plus récemment, aux États-Unis et au Canada. Les humains peuvent contracter l'infection à partir d'aliments d'origine animale contaminés par l'organisme infectieux. Les familles terriennes sont particulièrement à risque d'être contaminées par contact avec les animaux infectés ou en buvant du lait non-pasteurisé. Chez les bovins, les symptômes comprennent une diarrhée de profuse à sanguinolente, une diminution de la production de lait, de la pyrexie, de l'anorexie, de la déshydratation et de la dépression. L'infection peut se transformer en salmonellose septicémique et à la nécropsie, on peut observer une entérocolite fibrinonécrotique. L'infection survient plus souvent à la saison du vêlage qu'à d'autre moment. Les bovins en parc d'engraissement et les porcs peuvent aussi être affectés. Un état prolongé de porteur et d'excréteur du pathogène peut survenir. Chez l'homme, les symptômes consistent en diarrhée, fièvre, maux de tête, nausées, douleurs abdominales, vomissements et plus rarement du sang dans les matières fécales. Les souches de *Salmonella typhimurium* DT 104 résistent fréquemment à l'ampicilline, au chloramphénicol, à la streptomycine, aux sulfamides et à la tétracycline.

(Traduit par docteur André Blouin)

Can Vet J 1998; 39: 559–565

Health Canada, OIÉ Reference Laboratory for Salmonellosis, 110 Stone Road West, Guelph, Ontario N1G 3W4 (Poppe); Animal Health Laboratory, University of Guelph, Box 3612, Guelph, Ontario N1H 6R8 (Smart); National Laboratory for Enteric Pathogens (Khakhria, Johnson) and Bureau of Infectious Diseases (Spika), Laboratory Centre for Disease Control, Health Canada, Tunney's Pasture, Ottawa, Ontario K1A 0L2; Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 (Prescott).

Correspondence and reprint requests to: Dr. Cornelius Poppe, OIÉ Reference Laboratory for Salmonellosis, Health Canada, Guelph Laboratory, 110 Stone Road W., Guelph, Ontario N1G 3W4; Tel: 519-822-3300; Fax: 519-822-2280; e-mail: cornelius_poppe@hc-sc.gc.ca.

This paper has been peer-reviewed.

Infections with *Salmonella typhimurium* DT104, commonly resistant to the antimicrobials ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT), are occurring with increasing frequency in humans and animals in Europe, the United States, and Canada. This strain of *S. typhimurium* is recognized to be particularly virulent in animal and human hosts, and may be expected to be identified with increasing frequency in this country. This paper summarizes the existing understanding of the epidemiology of this infection.

Occurrence in animals and humans

The number of cases of *S. typhimurium* DT104 infection in humans in England and Wales rose from 259 in 1990 to 4006 in 1996 (1). This strain is now the 2nd most common *Salmonella* species isolated from humans in England and Wales, exceeded only by *S. enteritidis* PT4 (1–3). *Salmonella typhimurium* DT104 isolates from humans in England and Wales resistant to ACSSuT increased from 27.4% of the 259 isolates in 1990 to 54.1% of the 3837 isolates in 1995 (Table 1). Strains additionally resistant to trimethoprim (Tm) increased from 0% of the isolates in 1990 to 26.9% in 1995, and additionally resistant to ciprofloxacin (Cp) from 0% in 1990 to 6.2% in 1995. In total, 87.2% of these DT104 isolates were resistant to ACSSuT, or to ACSSuT and Tm or Cp in 1995. Other resistance patterns were found in 9.9% of the strains (4). In 1996, 58% of the DT104 strains were resistant to ACSSuT, the percentage of strains additionally resistant to trimethoprim declined slightly to 21%, while additional resistance to ciprofloxacin rose to 13%. One percent of DT104 isolates were resistant to ACSSuT and CpTm (1).

The number of *S. typhimurium* isolates from adult cattle, calves, sheep, pigs, chickens, turkeys, duck, geese, and game birds in England and Wales rose from 1397 in 1993 to 2264 in 1996; the number of DT104 strains rose from 458 (32.8% of the *S. typhimurium* isolates) in 1993 to 1513 (66.8%) in 1996. Most DT104 strains isolated in 1996 from cattle, sheep, pigs, and poultry had the resistance pattern ACSSuT, 9% were resistant to nalidixic acid (Nal), and 16% were resistant to Tm (5). Among 244 DT104 isolates from animals, their environment, and animal products in Northern Ireland in 1995–96, 243 showed multiresistance (6).

While infections have occurred in many species, *S. typhimurium* DT104 is primarily a pathogen of cattle (5–9). For example, in 1996, in England and Wales, *S. typhimurium* was the most frequently isolated serovar from calves, adult cattle, and pigs, and phagetype DT104 was the most common phagetype among the *S. typhimurium* isolates from each of these animal species. Thus, 71.3% of 1031 *S. typhimurium* strains from calves were DT104, 79.9% of 557 strains from adult cattle, and 40.3% of 283 *S. typhimurium* strains from pigs were DT104 (5). Similarly, in Scotland, 302 of 450 (67.1%) *Salmonella* isolates from 9 animal species during 1994–95 were *S. typhimurium* DT104 and almost all were multiply resistant; 90% of these isolates were from cattle (9). In Britain, since 1990, the number of *S. typhimurium* isolations from pigs has increased rapidly and, since 1992, DT104 has become the most frequently isolated phagetype (10). Although mostly associated with cattle and pigs, infections have occurred in a Noah's ark of animals, including chickens, turkeys, sheep, cats, horses, goats, dogs, emus, elk, coyotes, ground squirrels, rabbits, raccoons, chipmunks, mice, rats, foxes, badgers, and wild birds (5–13).

Originally recognized in Britain, the number of isolations has increased rapidly in Europe (14,15). In Germany, the isolation rate of DT104 from humans has increased since 1990. Analysis of the proliferation of the infection in different species showed that DT104 strains spread from cattle to pigs and humans (14). In France, increasing numbers of *S. typhimurium* strains isolated from cattle were resistant to ACST. The phenotype ACST was frequently associated with resistance to quinolones or sulfamethoxazole-trimethoprim (15). Among 182 *S. typhimurium* strains isolated in France in 1994, 76% of the human and 73% of the animal strains were resistant to the antimicrobials ACSSuT (16), which is highly suggestive that these isolates were DT104 (Table 1).

In the United States, isolations have also increased recently. *Salmonella typhimurium* resistant to ACSSuT was absent in Washington state prior to 1986, but rose from 13% of *S. typhimurium* cattle isolates in 1986–91 to 64% of isolates in 1992–94. A little later, similar increase of isolation rates of *S. typhimurium* resistant to ACSSuT was observed in humans in Washington state: only 2 of 46 human *S. typhimurium* isolates were resistant to ACSSuT in 1989 compared with 42.5% of 188 human isolates of *S. typhimurium* in 1994 (8). Nationwide, only 0.6% of human *S. typhimurium* strains isolated in 1979–80 and 7% of strains isolated in 1990 were resistant to ACSSuT (17,18). In 1995, *S. typhimurium* was the 2nd most commonly reported *Salmonella* serovar isolated from humans after *S. enteritidis* (13), accounting for 9702 (24%) of 41 222 cases of *Salmonella* infection (18). In the same year, the ACSSuT resistance pattern was present in 273 (28%) of a national sample of 976 *S. typhimurium* human isolates tested, again suggesting that these were DT104 (18). Unfortunately, phage typing is not commonly performed in the United States (8). However, of 30 phage-typed *S. typhimurium* strains with the resistance pattern ACSSuT isolated in 10 states in 1995, 25 (83%) were DT104 (18); and, 39 of 43 (90.7%) *S. typhimurium* strains resistant to ACSSuT isolated during 1994–96 were DT104 (17). In 1996, the ACSSuT resistance pattern was present in 32% of 282 human *S. typhimurium* isolates tested at the US Department of Health and Human Services, Centres for Disease Control and Prevention (CDC) (18). Of 549 *S. typhimurium* isolates collected from animals in 1997, 26% showed the ACSSuT resistance pattern and 90 of these strains (63%) were identified as DT104. Outbreaks were observed in dairy herds and feedlot cattle in the Pacific northwest, in dairy herds in New York state and Vermont, and in swine in Nebraska (13).

In Canada, 59 and 148 cases of human *S. typhimurium* DT104 infection were recognized in 1995 and 1996, respectively; no deaths occurred. In 1993–96, 41% of 544

Table 1. Occurrence and drug resistance of *Salmonella typhimurium* DT104

Country or Region	Year(s)	Species	No. of *S. typhimurium* isolates	No. of DT104 strains	Antibiotic Resistance (% of DT104 or of *S. typhimurium* strains)	Reference(s)
England and Wales	1990	Human	5472	259	27.4% DT104: ACSSuT	(1–4)
	1995	"	6976	3837	54.1% DT104: ACSSuT; 26.9% ACSSuT + Tm[a]; 6.2% ACSSuT + Cp[b]	(1–4)
	1996	"		4006	58% DT104: ACSSuT; 21% ACSSuT + Tm; 13% ACSSuT + Cp	(1)
	1993	Animal	1397	458		(5)
	1996	"	2264	1513	Most DT104:ACSSuT; 9% Nal[c]; 16% Tm	
Scotland	1994–1995	Bovine (90% of isolates), 8 other animal species	450	302	98% DT104: ACTSp[d]	(9)
Northern Ireland	1995–1996	Animal and environmental	353	244	99.6% DT104: Multiresistant	(6)
France	1994	Human	82	ND[e]	76% ST[f]: ACSSuT	(16)
		Animal	100	ND	73% ST: ACSSuT	
Washington state	1986–1991	Bovine	83	ND	13% ST: ACSSuT	(8)
	1992–1995	"	51	ND	64% ST: ACSSuT	
	1989	Human	46	ND	4.3% ST: ACSSuT	
	1994	"	188	ND	42.5% ST: ACSSuT	
USA	1990	Human			7% ST: ACSSuT	(17,18)
	1995	"	976	ND	28% ST: ACSSuT	
	1996	"	282	ND	32% ST: ACSSuT	
	1997	Animal	549	ND	26% ST: ACSSuT	(13)
			143	90	100% DT104: ACSSuT	
Canada	1993–1996	Human	544	221		(unpublished)
	1995	"			46.0% DT104: ACSSuT	
	1997	"			57.6% DT104: ACSSuT	
	1996	Animal, food, environmental	202	69	85.5% DT104: ACSSuT or ACSuT	(unpublished)
	1997	"	309	118	94.2% DT104: ACSSuT or ACSuT	

[a]Trimethoprim
[b]Ciprofloxacin
[c]Nalidixic acid
[d]Spectinomycin
[e]Not Determined
[f]*Salmonella typhimurium*

human *S. typhimurium* strains isolated and examined at the Health Canada, Laboratory Centre for Disease Control (LCDC) were phage-typed as DT104, and the resistance of the DT104 strains to ACSSuT increased from 46% (18 of 39) of strains in 1995, to 57.5% (76 of 132) of strains in 1997 (unpublished observations). During 1996, 34.2% (69 of 202) of *S. typhimurium* isolates from animals, food and environmental sources were typed as DT104 at the Health Canada, OIÉ *Salmonella* Reference Laboratory. During the first 10 mo of 1997, 38.2% (118 of 309) *S. typhimurium* isolates from animals were DT104, suggesting that this type is still increasing in number. Eighty-six percent (59 of 69) of the DT104 strains in 1996 and 94.2% (49 of 52) of these strains in 1997 were resistant to ACSSuT or ACSuT (unpublished observations) (Table 1).

Details of the frequency of DT104 isolations in continents other than Europe and North America are not readily available.

Relationship between infections in animals and humans

There is a clear association between infections of farm animals, or foods of animal origin, and human infection, showing that DT104 readily infects people in contact with infected animals or their products (19–23). For example, a Canadian study described the isolation of DT104 from calf-rearing facilities, veal operations and dairies, and from horses and humans (19). The outbreaks occurred in calves and heifers purchased from auction markets in Alberta and British Columbia. The organism caused an outbreak of salmonellosis in heifers, subsequently contaminating 2 veterinary clinics and infecting an animal health technician (19). As another example, 4 members of a farm family in Canada became infected after contact with infected calves (24).

In Britain, a study of DT104 isolations identified 6 separate associations of infection between farming families and their livestock (20). Five involved calves or dairy

cows and one involved sheep. In 4 of these associations, the isolations from animals and humans were closely related, temporally. However, the other 2 infections occurred 8 to 9 mo earlier in humans than in animals on the same farm. It was suggested that one farmer became infected by fecal-oral contamination, or through saliva or other direct contact with infected calves (20,22). In another British study of DT104 infection in cattle, possible or confirmed human illness occurred in farm workers or farm families on 20% of affected farms (11). Veterinarians may be occupationally exposed; DT104 was isolated from pustules on the arm of a veterinarian who assisted in a calving (25). Indirect transmission to farm families may result from inadequate hand washing or from wearing inadequately disinfected footwear or working overalls indoors (20). The organism may also be spread by farming equipment (26).

Cats and dogs may sometimes be a source for human infection (26,27). *Salmonella* may be shed in large numbers from the mouth of asymptomatic cats, so that grooming cats may contaminate their coats (27).

Drinking unpasteurized milk by farm families may be another source of human infection (20). Strain DT104 was shed persistently from one quarter of the udder of a cow in England (28). The drinking of unpasteurized milk is a common practice among farm families in Ontario (29), which may put these families at particular risk from this virulent strain.

Food-borne outbreaks of *S. typhimurium* DT104 have been associated with the consumption of pork sausages, chicken, meat paste (21), beef (23), and soft cheese (13). Pigs that are asymptomatic long-term carriers may contaminate the meat at slaughter (30). A case control study of infection with *S. typhimurium* DT104 in England and Wales showed that food-borne infections were more common than infections resulting from contact with animals (21). Among 83 cases of infection with DT104, 70 (84%) were significantly associated with the consumption of foods and 13 (16%) reported contact with ill animals; of the latter group, 5 had contact with cattle infected with *Salmonella*, 4 with calves with diarrhea, and 4 with ill pets (21).

Clinical characteristics of the infection in animals and humans

In cattle, in Alberta, DT104 was most commonly recognized in 2- to 4-week-old "poor doing" calves suffering from chronic diarrhea (19). Calves that were recently purchased and had travelled over considerable distances became sick soon after arrival and experienced a mortality of 40% to 50%. At necropsy, the calves had fibrinonecrotic enterocolitis. In heifers, infection was characterized by dysentery (19). Infections of adult females and their offspring occurred most frequently during the calving or lambing season (11,20). In some outbreaks, infection of lambs has occurred on the same farms where the strain was recently isolated from cattle (25). In dairy cattle, prominent symptoms of infection were pyrexia, diarrhea, and a decrease in milk production (28). Fecal carriage is persistent after outbreaks of this type of salmonellosis, since the organism has been recovered from feces for up to 18 mo after infection (11). This unusual persistence is important, since the introduction of asymptomatic carrier animals has resulted in disease in previously unaffected animals (31).

Despite the virulence, persistence, and ready transmission of the infection, a case control study in Britain found that in cattle, most outbreaks lasted for less than a week and that less than 4% of animals within herds were clinically affected (7,11). Cases were more common in summer than winter and the incidence was greater in large herds than in small ones. The incidence of disease was about 33% in calves, compared with only 4% of adults in affected herds. The index case was most often a young calf. The most common clinical signs were watery diarrhea, loss of appetite, and loss of condition. Abortion was reported rarely. Although the incidence was low, about 40% of clinically affected cattle died, and even higher fatality rates have occurred in calves. Subclinical carriage was common and persisted for up to 18 mo. An increased risk of disease was associated with cattle obtained from dealers, the introduction of newly purchased cattle to the farm, with housing rather than being at pasture, and with lack of isolation facilities. Wild birds and cats were recognized as possible vectors and access to cattle feed by wild birds was associated with increased risk of disease. Contamination of feed, grain stores, and bedding by feces from infected wildlife were also common (7,11).

Salmonellosis may be more severe when the host is concomitantly infected with other pathogens. For example, in a group of 30 pregnant dairy heifers that showed clinical signs of acute bovine viral diarrhea infection, one heifer developed severe diarrhea but no *Salmonella* could be isolated from the feces. However, on necropsy, DT104 was isolated from the heifer's lung, spleen, kidney, and ileum (32).

Nine-week-old feeder pigs infected with *S. typhimurium* DT104 had diarrhea and vomited, and some pigs died suddenly. Upon necropsy, fibrinonecrotic enterocolitis, enlarged mesenteric lymph nodes, and splenomegaly were observed. The organism was isolated from the colon, ileum, jejunum, mesenteric lymph nodes, and the spleen (unpublished observations). Subclinical infection of pigs is widespread in England and Wales (30).

Clinical signs of DT104 salmonellosis in cats included severe gastroenteritis with vomiting, bloody diarrhea, fever, anorexia, dehydration, and depression for 4 to 10 d (12,33). Intermittent diarrhea persisted for 1 mo and fecal shedding lasted for more than 14 wk (12,33). The characteristic and unusually persistent excretion by cattle, horses, cats, or other animal species increases the risk for further dissemination of the pathogen (28).

The most common symptoms in humans infected by DT104 were diarrhea (100%), fever (80%), abdominal pain (65%), vomiting (45%), and blood in the stool (27%) (19,20). An outbreak of diarrheal illness among elementary school children in Nebraska resulted in 19 (59%) of 32 children developing diarrhea; other symptoms observed among the children having diarrhea were fever (89%), headache (89%), nausea (89%), vomiting (58%), and bloody diarrhea (16%). None required hospitalization. Culture of stool samples from 7 children all yielded *S. typhimurium* DT104 (18). A study at the CDC showed that *S. typhimurium* strains of the R-type

ACSSuT were more invasive in humans than were other *S. typhimurium* strains, in that 13% of the R-type ACSSuT strains were blood isolates compared with only 4% of the *S. typhimurium* strains that were not of this R-type (13). In England and Wales, 41% (34 of 83) of DT104-infected patients were hospitalized and 3% of 295 patients died (21); this is a significantly greater hospitalization and death rate than in other *S. typhimurium* infections.

Although *S. typhimurium* DT104 appears to be more virulent than other phage types of *S. typhimurium*, the reason for this apparent enhanced virulence is unknown; it may be related to the ability of the organism to better colonize the host or persist for longer in a colonized host.

Resistance of *S. typhimurium* DT104 to antibiotics

Salmonella typhimurium DT104 is unusual in that multiple antibiotic resistance is a common characteristic of this strain. The reasons for the apparent readiness of this phagetype to become resistant to antibiotics is unknown and more work needs to be done to understand its apparent enhanced ability to acquire antibiotic resistance genes. There are marked differences in the ability of certain *Salmonella* serovars and phagetypes to acquire genes encoding resistance to antibiotics. Although *S. enteritidis* has been isolated frequently from poultry and humans in many countries, the isolates have, with the exception of infrequently isolated strains and phagetypes, such as PT24 (34), remained mostly sensitive to antibiotics (5,35). Similarly, *S. dublin*, although having been isolated frequently from the same cattle population in England and Wales as *S. typhimurium* DT104 and undoubtedly having been exposed to the same antibiotics, has remained sensitive to drugs (5). Unlike *S. typhimurium* DT204c, another cattle-associated pathogen in which resistance to ACSSuT is encoded by plasmids (36), the genes encoding resistance to ACSSuT in *S. typhimurium* DT104 strains, although possibly of plasmid origin, have become chromosomally integrated (37). Ridley and Threlfall (38) and Sandvang et al (39) examined integron-mediated multiple antibiotic resistance genes in DT104 strains and found integron "hot spots", usually in 2 copies, in the majority of strains expressing resistance to ACSSuT or SSuSp (Sp = spectinomycin), but not in those resistant to SSu. Amplicons of 1 kb from strains resistant to ACSSuT, or resistant to at least SSuSp, revealed the presence of *aadA* (aminoglycoside adenyltransferase A) genes encoding streptomycin and spectinomycin resistance. Amplicons of approximately 1.2 kb from strains expressing ACSSuSpT or ASu resistance encoded the *pse*-1 β-lactamase gene. Mutations were detected in the *gyrA* sequences encoding ciprofloxacin resistance (38,39).

Examination of DT104 strains which are additionally resistant to trimethoprim (Tm) demonstrated that such resistance was encoded by a nonconjugative but mobilizable plasmid of 4.6 Mda, which also encoded resistance to sulfonamides (40). Resistance to apramycin and gentamicin (both aminoglycosides) is rare, but it is plasmid-mediated (9). Examination of the genes encoding ampicillin resistance from 182 *S. typhimurium* strains resistant to ACSSuT, isolated in France in 1994, revealed that 20.7% and 22% of 82 human and 100 animal isolates, respectively, were of the TEM β-lactamase type, and 73.2% and 77% of the human and animal strains, respectively, were of the CARB β-lactamase type (16).

Prevention and control

Prevention of the infection in humans requires safely storing and handling food (by consumers and food service establishment personnel), washing hands, wearing gloves, and adequately cooking meat and poultry products (41). Drinking unpasteurized milk by farm families and others should be actively discouraged, and dairies should ensure the adequate pasteurization of milk (29,42,43). Eating soft and other cheese made from inadequately pasteurized milk should also be discouraged (13,43). Veterinarians should consider the zoonotic nature of the disease and advise their clients accordingly.

Ciprofloxacin has been employed as the initial treatment for enteric fever in the United Kingdom (44) and other countries (45), and fluoroquinolones have been used in many countries to treat nontyphoid infections in humans and animals (46). This has led to the increasing number of isolations of fluoroquinolone-resistant *Salmonella* from human (1,4,45) and animal sources (46,47). Such evidence should encourage health care professionals to prescribe and use the fluoroquinolones and other antibiotics prudently and sparingly. In the United States, the recent approval of sarafloxacin to control mortality in broiler chickens and growing turkeys associated with *E. coli* infections may result in increased isolation of DT104 strains additionally resistant to the fluoroquinolones. The use of fluoroquinolones in food-producing animals, particularly in cattle, should be avoided (41).

Salmonella typhimurium DT104 infections in cattle may be prevented by purchasing replacement stock directly, rather than via livestock dealers, by maintaining a 4-week quarantine period of purchased cattle, by housing sick animals in dedicated isolation areas, and by preventing wild birds from having access to feed for cattle (7,11). Cattle and their owners may be contaminated with *S. typhimurium* or they may acquire the infection by attending cattle shows and visiting sales barns and auction markets. Although vaccination with killed vaccines (bacterins) does not usually produce an effective immune response against *Salmonella* (48), Evans and Davies (11) reported that vaccination with a bacterin led to rapid cessation of excretion of the organism in 7 out of 7 dairy herds, whereas 5 of 5 nonvaccinated herds were subclinically affected for at least 6 mo and 2 of them experienced recurrence of subclinical infection after 2 y (11). Vaccination against DT104 has become fairly common practice in Britain. Basic management procedures for the prevention and control of the infection in pigs consist of such measures as supplying *Salmonella*-free stock; an all-in, all-out system for rearing and finishing operations; using disinfectant foot dips between separate buildings; and the proper and discriminate use of disinfectants (30).

In Canada, effective ways are required to ensure that rendered animal waste products used in the production of animal feed are maintained as sterilized products, so that the well-recognized cycle of *Salmonella* infections

in intensively reared food animals is broken. In addition, surveillance to prevent the introduction of infected live food animals, such as poultry, into the country would be helpful, if combined with development of policies that prevent the use of these animals for breeding or for food. The development and application of Hazard Analysis Critical Control Point (HACCP) plans and procedures on the farm and throughout the food production chain will undoubtedly aid in the production of milk, beef, pork, chicken, and other food products of animal origin that are free of or carry a reduced number of pathogens, such as *S. typhimurium* DT104. Continued surveillance will not only determine the frequency of infection and contamination, but it will also aid in determining the effect of control measures to reduce infection and contamination and, hopefully, in improving the health of the public and those who care for animals.

In conclusion, *S. typhimurium* DT104 has emerged as an important multidrug-resistant *Salmonella* strain, associated particularly with cattle. This strain is being increasingly found in other species and is associated with foodborne illness in people. Veterinarians in Canada should be aware of the potential of this strain to cause serious disease in animals and in the people working with these animals. Measures to limit the spread of this infection will depend on the united efforts of veterinarians, farmers, and the public health authorities. CVJ

References

1. Threlfall EJ, Ward LR, Rowe B. Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella typhimurium* DT104 in England and Wales. Eurosurveillance. European Commun Dis Bull 1997; 2: 81–84.
2. Anon. *Salmonella typhimurium* infections in humans — United Kingdom. Canada Commun Dis Rep 1996; 22: 119.
3. Anon. *Salmonella* infections, England and Wales. Commun Dis Reports CDR Wkly 1990–1991; 90/01–91/03.
4. Threlfall EJ, Frost JA, Ward LR, Rowe B. Increasing spectrum of resistance in multiresistant *Salmonella*. Lancet 1996; 347: 1052–1053.
5. Ministry of Agriculture, Fisheries and Food, Welsh Office Agriculture Department, Scottish Office, Agriculture and Fisheries Department. *Salmonella* in livestock production — 1996. Available from: Epidemiology Department, Central Veterinary Laboratory, New Haw, Addlestone, Surrey, KT15 3NB, UK.
6. McDowell SWJ, Spence A. Antimicrobial resistance in *Salmonella* isolates from animal sources in Northern Ireland. Irish Vet J 1997; 50: 364.
7. Evans SJ. A case control study of multiple resistant *Salmonella typhimurium* DT104 infection of cattle in Great Britain. Cattle Pract 1996; 4: 259–266.
8. Besser TE, Gay CC, Gay JM, et al. Salmonellosis associated with *S. typhimurium* DT104 in the USA. Vet Rec 1997; 140: 75.
9. Low JC, Angus M, Hopkins G, Munro D, Rankin SC. Antimicrobial resistance of *Salmonella enterica* Typhimurium DT104 isolates and investigation of strains with transferable apramycin resistance. Epidemiol Infect 1997; 118: 97–103.
10. Gresham ACJ. An old disease of increasing significance — Salmonella infection in pigs in Great Britain. Pig J 1996; 37: 59–64.
11. Evans S, Davies R. Case control study of multiple-resistant *Salmonella typhimurium* DT104 infection of cattle in Great Britain. Vet Rec 1996; 139: 557–558.
12. Low JC, Tennant B, Munro D. Multiple-resistant *Salmonella typhimurium* DT104 in cats. Lancet 1997; 348: 1391.
13. Miller, MA. Widespread emergence in the United States of multiple drug-resistant type of *Salmonella typhimurium*. FDA Veterinarian 1997; 12 (4): 5–6.
14. Prager R, Liesegang A, Streckel W, et al. *Salmonella enterica*, serovar Typhimurium, phage type DT104 the emerging epidemic clone in Germany. In: Proc 4th Int Meet Bacterial Epidemiological Markers, Elsinore, Denmark, 10–13 September 1997; p. 104.
15. Moury F, Brisabois A, Fremy S. Antibiotic susceptibility of *Salmonella* strains isolated from animals and their environment. In: Proc 4th Int Meet Bacterial Epidemiological Markers, Elsinore, Denmark, 10–13 September 1997; p. 138.
16. Brisabois A, Cazin I, Breuil J, Collatz E. Surveillance of antibiotic resistance in Salmonella. Eurosurveillance. European Commun Dis Bull 1997; 2: 19–20.
17. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. New Engl J Med 1998; 338: 1333–1338.
18. Hosek G, Leschinsky D, Irons S, Safranek TJ. Multidrug-resistant *Salmonella* serotype Typhimurium — United States, 1996. Morb Mort Weekly Rep 1997; 46: 308–310.
19. Schoonderwoerd M, Fenton R, Stone W. *Salmonella typhimurium* infection in Alberta livestock. In: 38th Conf Can Lab Workers Anim Dis, May 31–June 1, 1988.
20. Fone DL, Barker RM. Associations between human and animal infections with *Salmonella typhimurium* DT104 in Herefordshire. Commun Dis Rep CDR Rev 1994; 4: R136–R140.
21. Wall PG, Morgan D, Lamden K, et al. A case control study of infection with an epidemic strain of multiresistant *Salmonella typhimurium* DT104 in England and Wales. Commun Dis Rep CDR Rev 1994; 4: R130–R135.
22. Wall PG, Morgan D, Lamden K, et al. Transmission of multiresistant strains of *Salmonella typhimurium* from cattle to man. Vet Rec 1995; 136: 591–592.
23. Davies A, O'Neill P, Towers L, Cooke M. An outbreak of *Salmonella typhimurium* DT104 food poisoning associated with eating beef. Commun Dis Rep CDR Rev 1996; 6: R159–R162.
24. Lior H, Khakhria R. Salmonellae, Shigellae, enteropathogenic *E. coli*, Campylobacters and *Aeromonas* identified in Canada. Monthly Rep, 1988–1989. Health Canada, National Enteric Reference Centre, Laboratory Centre for Disease Control, Ottawa, Canada.
25. Anon. Isolations of *Salmonella typhimurium* DT104 continue to increase. Vet Rec 1997; 140: 668–671.
26. Anon. Increase in *S. typhimurium* DT104 infections in cattle. Vet Rec 1997; 140: 5–7.
27. Wall PG, Threlfall EJ, Ward LR, Rowe B. Multiresistant *Salmonella typhimurium* DT104 in cats: a public health risk. Lancet 1996; 348: 471.
28. Sharp MW, Rawson BC. Persistent *Salmonella typhimurium* DT104 infection in a dairy herd. Vet Rec 1992; 131: 375–376.
29. West AM, Martin SW, McEwen SA, Clarke RC, Tamblyn SE. Factors associated with the presence of *Salmonella* spp. in dairy farm families in Southwestern Ontario. Can J Public Health 1988; 79: 119–123.
30. Davies R, Wray C. Study of multi-resistant *Salmonella typhimurium* infection in pig herds: preliminary findings. Pig J 1997; 40: 80–88.
31. Low JC, Hopkins G, King T, Munro D. Antibiotic resistant *Salmonella typhimurium* DT104 in cattle. Vet Rec 1996; 138: 650–651.
32. Penny CD, Low JC, Nettleton PF, et al. Concurrent bovine viral diarrhoea virus and *Salmonella typhimurium* DT104 infection in a group of pregnant dairy heifers. Vet Rec 1996; 138: 484–489.
33. Wall PG, Davis S, Threlfall EJ, Ward LR, Ewbank AJ. Chronic carriage of multidrug resistant *Salmonella typhimurium* in a cat. J Small Anim Practice 1995; 36: 279–281.
34. Frost JA, Ward LR, Rowe B. Acquisition of a drug resistance plasmid converts *Salmonella enteritidis* phage type 4 to phage type 24. Epidemiol Infect 1989; 103: 243–248.
35. Rodrique DC, Cameron DN, Puhr ND, et al. Comparison of plasmid profiles, phage types, and antimicrobial resistance patterns of *Salmonella enteritidis* in the United States. J Clin Microbiol 1992: 854–857.
36. Threlfall EJ, Brown DJ, Rowe B, Ward LR. Occurrence of *S. typhimurium* DT204c in poultry in England and Wales. Vet Rec 1990; 127: 234.
37. Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle and humans of *Salmonella typhimurium* DT104 with chromosomally integrated multiple drug resistance. Vet Rec 1994; 134: 577.

38. Ridley AM, Threlfall EJ. Molecular epidemiology of integron-associated multiple antibiotic resistance genes in epidemic *Salmonella typhimurium* DT104 in England and Wales. In: Proc 4th Int Meet Bacterial Epidemiological Markers, Elsinore, Denmark, 10–13 September 1997; p. 24.
39. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol Lett 1998; 160: 37–41.
40. Threlfall EJ, Hampton MD, Schofield SL, Ward LR, Frost JA, Rowe B. Epidemiological application of differentiating multiresistant *Salmonella typhimurium* DT104 by plasmid profile. Commun Dis Rep CDR Rev 1996; 6: R155–R159.
41. Hollingworth J, Kaplan B. Federal agencies collaborate to control dangerous new *Salmonella* strain. J Am Vet Med Assn 1997; 210: 1715–1716.
42. Ryan CA, Nickels MK, Hargrett-Bean NT, et al. Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. J Am Med Assn 1987; 258: 3269–3274.
43. Bezanson GS, Khakhria R, Duck D, Lior H. Molecular analysis confirms food source and simultaneous involvement of two distinct but related subgroups of *Salmonella typhimurium* bacteriophage type 10 in major interprovincial *Salmonella* outbreak. Appl Environ Microbiol 1985; 50: 1279–1284.
44. Rowe B, Ward LR, Threlfall EJ. Ciprofloxacin and typhoid fever. Lancet 1992; 339: 740.
45. Wain J, Hoa NTT, Chinh NT, et al. Quinolone-resistant *Salmonella typhi* in Vietnam: Molecular basis of resistance and clinical response to treatment. Clin Infect Dis 1997; 25: 1404–1410.
46. Wray D, McLaren I, Wise R, Piddock LJV. Nalidixic acid-resistant Salmonellae. Vet Rec 1990; 126: 489.
47. Griggs DJ, Hall MC, Jin YF, Piddock LJV. Quinolone resistance in veterinary isolates of *Salmonella*. J Antimicrob Chemother 1994; 33: 1173–1189.
48. Smith BP, Habasha FG, Reina-Guerra M, Hardy AJ. Immunization of calves against salmonellosis. Am J Vet Res 1980; 41: 1947–1951.

BOOK REVIEW

COMPTE RENDU DE LIVRE

Rooney JR, Robertson JL. ***Equine Pathology.*** Iowa State University Press, Ames, Iowa, 1996. 482 pp ISBN 0-8138-2334-X. $119.95 plus shipping and handling.

Equine Pathology is a concise, informative text written by 2 men, each with a lifelong interest in diseases of the horse. From the Introduction, it is clear to the reader that this book is, "not intended to replace other texts of general or special pathology," rather, "to bring to the reader what has been done and what is known about that species (the horse) without too much unwarranted speculation based on studies in other species." Literature in English, German, and French has been reviewed. The authors are students of history, as well as pathology. Diseases that were familiar to the ancient Greeks are still important to pathologists and clinicians today. An interesting reference that is quoted several times is the incidence of disease recorded between 1886 and 1895 in Prussian cavalry and artillery horses, a population of about 100 000 animals. North American pathologists practising today can feel confident when Drs. Rooney and Robertson say a disease is rare. They have necropsied about 10 000 light horses in the past 30 y.

The book is divided into 20 chapters. Most chapters deal with a single body system (cardiovascular, respiratory, gastrointestinal, etc.). Dr. Rooney's interest in lameness and biomechanics is evident. There are 5 chapters dedicated to the musculoskeletal system under the headings of Locomotor System (General), Foreleg, Rear Leg, Vertebral Column, and Stability Theory and Pathogenesis of Lameness. There are also chapters on Shock, Autopsy Methods, and Forensics. The discussion of each disease varies in length from a few paragraphs to 2–3 pages. Although some of the discussions are brief, they are valuable as a review or as an adjunct to a more specialized text or recent journal article. The book is illustrated with ample, well-captioned, gross and microphotographs; radiographs; and schematics. As with any text book, there are instances where new information has come to light since the book was published. For example, we have gained more understanding of the epidemiology of equine protozoal myeloencephalitis since this book went to press. However, the information given in the chapter on Stability Theory and Pathologenesis of Lameness is not found in any other book in my library. The autopsy method is clearly described with text, schematics, and photographs. The instruction is excellent for either an experienced pathologist as a review or a veterinary student as a how-to guide. The chapter on forensics discusses the responsibilities of a usual witness and an expert witness. The importance of professionalism and chain-of-custody and complete, accurate reports is emphasized. A brief discussion on sudden death in several situations follows.

Equine pathology will never replace my constant companions, the 3 volumes of the 4th edition of The Pathology of Domestic Animals (Jubb, Kennedy and Palmer, 1993). However, this text will take its place beside them as my equine reference. Often, the authors' own opinions, although usually well backed up by experience or reference (the bibliography is 65 pages long), can be heard. This made the book all the more entertaining and thought provoking. It made me want to meet the authors in person for further discussions.

Reviewed by ***Dr. Jiggs Gough,*** *Pathologist, Laboratory Head, Animal Health Laboratory, University of Guelph, Ridgetown, Ontario N0P 2C0.*

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Canada Communicable Disease Report CCDR

Volume 24-03
1 February 1998

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HUMAN ISOLATES OF *SALMONELLA TYPHIMURIUM* DT104 IN ONTARIO

Introduction

Multidrug-resistant isolates of *Salmonella typhimurium* known as definitive type 104 (DT104) have emerged as an important cause of salmonellosis in the United Kingdom[1,2] and the United States[3]. The most common resistance phenotype (R-type) among DT104 isolates is ACSSuT (resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline); isolates with this R-type have been found not only among humans, but among a wide range of mammals, poultry, cats, and dogs in the United Kingdom[1,2].

In Ontario, Canada, isolates of *S. typhimurium* represent 16% to 31% of all human *Salmonella* isolates received at the Central Public Health Laboratory, Toronto, for confirmation and identification of serotypes over a 4-year period between 1993 and 1996. In response to concerns regarding the epidemic spread of *S. typhimurium* DT104 R-type ACSSuT in the United Kingdom and United States, a selected number of Ontario isolates of *S. typhimurium* over a 3-month period, between 20 November 1996 and 21 January 1997, were investigated for antimicrobial susceptibility and the presence of phage type DT104.

Methods

Isolates: *S. typhimurium* isolates were submitted to the Central Public Health Laboratory from private community-based laboratories which provide services to physicians, nursing homes, clinics and hospitals across Ontario, and from regional public-health laboratories. The 115 isolates examined in this study were collected from patients aged 1 to 71 years. All isolates were from stools, except for two which were from urine samples.

Susceptibility testing: Antimicrobial agents were tested by agar dilution with Mueller-Hinton (M-H) agar at the following concentrations (mg/L): ampicillin 8, 16; chloramphenicol 8, 16; tetracycline 4, 8; ticarcillin 16, 64; piperacillin 16, 64; trimethoprim 8; sulfamethoxazole 256; cotrimoxazole (trimethoprim/sulfamethoxazole) 0.5/9.5, 2.0/38.0; gentamicin 4; tobramycin 4; amikacin 16; cephalothin 8, 16; cefoxitin 8, 16; ceftazidime 8, 16; cefotaxime 8, 32; ciprofloxacin 1, 2; nalidixic acid 4, 50. Susceptibility testing was carried out according to the method of the National Committee for Clinical Laboratory Standards[4]. For the susceptibility testing of trimethoprim, sulfamethoxazole, and cotrimoxazole, 5% lysed horse blood was added to the M-H agar.

Results and Discussion

One hundred and fifteen isolates were investigated for antimicrobial susceptibility. Of these, 49 isolates (42.6%) were resistant to one or more antimicrobial agents, and only one DT104 isolate was susceptible to all antimicrobial agents tested (Table 1). Thirty-six of the resistant isolates were identified as DT104 phage type. The R-type of 29 of the 36 *S. typhimurium* DT104 isolates was ACSSuT (Tic Pip) (resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, ticarcillin,

and piperacillin). One isolate was also resistant to nalidixic acid, and another was also resistant to trimethoprim and cotrimoxazole. The R-type of three isolates was ASu (Tic Pip) (resistance to ampicillin, sulfamethoxazole, ticarcillin and piperacillin) but these were susceptible to chloramphenicol, tetracycline, and streptomycin. One of the other two isolates was susceptible to all antimicrobial agents tested, and the other had the R-type SSu (resistance to streptomycin and sulfamethoxazole). Four isolates belonged to phage type 193, nine isolates to 193 variant, one isolate to 208, and one isolate to 10; three isolates were untypable. The 14 isolates were resistant to one to four antimicrobial agents (Table 1).

Table 2 shows the number of *S. typhimurium* DT104 by age group. The majority of *S. typhimurium* DT104 isolates were obtained from young children, 1 to 9 years of age, or from young adults, 20 to 44 years of age.

The vehicles of infection in the present study were not identified. Several studies in the United Kingdom[5-7] have indicated that multiple food vehicles are involved in the transmission of this organism to humans: sources such as raw milk, pork sausages, chicken, and meat paste. Although cattle are the main reservoir of *S. typhimurium* infection, only a few reports have shown direct transmission from cattle to humans[5-7]. Further epidemiologic studies are needed to determine the source of infection for humans in Ontario, in order that control measures be implemented to reduce the risk of contamination.

Table 1 Antimicrobial susceptibility pattern and phage type of 50 *S. typhimurium* isolates, Ontario, 20 November 1996 - 21 January 1997

R-type[a]	No. of isolates	Phage type
ACSSuT (Tic Pip)	29	104
ACSSuT (Tic Pip Nal)	1	104
ACSSuTTm (Tm/Su Tic Pip)	1	104
ASu (Tic Pip)	3	104
SSu	1	104
None[b]	1	104
T	5	193 variant
SSuT	1	193
T	3	193
A (Tic Pip)	1	208
CT	1	10
AT (Tic Pip)	1	untypable
CT	2	untypable

a) A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; Tic, ticarcillin; Tm, trimethoprim; Tm/Su (cotrimoxazole); ip, piperacillin; Nal, nalidixic acid.
b) One isolate was susceptible to all antimicrobial agents tested.

Table 2 Referred isolates of *S. typhimurium* DT104, by age group, Ontario, 20 November 1996 - 21 January 1997

Age group	No. (%) of isolates
0-9	13 (36.1)
10-19	6 (16.7)
20-44	12 (33.3)

45-64	2 (5.6)
65+	3 (8.3)

The present study also shows that the most common R-type is ACSSuT, as reported by other investigations[1,2,6]. Only one isolate showed resistance to trimethoprim and cotrimoxazole; it is noteworthy that this isolate was from the urine of a 2-year-old male child. None of the isolates examined in this study was resistant to the newer fluoroquinolones, although one isolate showed high level resistance (> 100 mg/L) to the first generation quinolone, nalidixic acid. This isolate was from a 71-year-old individual who may have had previous exposure to the drug. Speculation is that the emergence of trimethoprim and fluoroquinolone resistance among *S. typhimurium* DT104 is the result of the use of these agents in the treatment of infections in cattle[5,6].

It is interesting that, in this limited study, the majority of isolates were from young children, in non-outbreak (sporadic) situations. One could speculate that the reservoirs for DT104 among this group are probably food-related, or perhaps related to direct association with infected pets. The suspected animal source of an outbreak in the United States among elementary school children was difficult to confirm, since neither the ill kitten or turtle which the children had handled was available for testing for *S. typhimurium*[3].

From January to November 1997, 22.9% of all *Salmonella* isolates submitted to the Central Public Health Laboratory were *S. typhimurium*, with a peak of 28.4% in September (A Borczyk, Laboratory Services Branch, Toronto: personal communication, 1997). A more comprehensive study is being conducted at the Central Public Health Laboratory in order to determine the prevalence of *S. typhimurium* DT104 in Ontario.

Acknowledgements

The authors acknowledge the assistance of S. Lombardi for typing and Dr. F. Jamieson for reviewing the manuscript.

References

1. Threlfall EJ, Frost JA, Ward LR et al. *Increasing spectrum of resistance in multiresistant* ***Salmonella typhimurium***. Lancet 1996;347:1052-53.

2. Wall PG, Threlfall EJ, Ward LR et al. *Multiresistant* ***Salmonella typhimurium*** *DT104 in cats: a public health risk*. Lancet 1996;348:471.

3. Hosek G, Leschinsky D, Irons S et al. *Multiresistant* ***Salmonella*** *serotype* ***typhimurium*** *- United States, 1966*. JAMA 1997;227:1513.

4. National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 3rd ed. Approved Standard. Villanova, PA: National Committee for Clinical Laboratory Standards, 1993, [NCCLS document no. M7-A3 (Vol. 13, No. 25)].

5. Wall PG, Morgan D, Lamden K et al. *A case control study of infection with an epidemic strain of multi-resistant* ***Salmonella typhimurium*** *DT104 in England and Wales*. CDR (Lond Engl Rev) 1994;R130-35.

6. Davies A, O'Neill P, Towers L et al. *An outbreak of* ***Salmonella typhimurium*** *DT104 food poisoning associated with eating beef*. CDR (Lond Engl Rev) 1996;R159-62.

7. Fone DL, Barker RM. *Associations between human and farm animal infections with **Salmonella typhimurium** DT104 in Herefordshire*. CDR (Lond Engl Rev) 1994;R136-39.

Source:

N Harnett, PhD, Dip Bact, Research Scientist, J Wan, BSc, RT, AIMLS, Technologist, V Brunins, BSc, RT, Senior Technologist, Enteric Reference Laboratory; A Borczyk, MSc, Chief, Enteric and Special Procedures Laboratories, Clinical Bacteriology Section, Central Laboratories, Laboratory Services Branch, Ontario Ministry of Health, Toronto; R Khakhria, BSc, Head Phage Typing, Molecular Epidemiology and Surveillance Section, National Laboratory for Enteric Pathogens, Bureau of Microbiology; W Johnson, PhD, Chief, National Laboratory for Bacteriology and Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, ON.

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Last Updated: 2002-11-08 ▲ Important Notices

1: Can Commun Dis Rep. 1998 Feb 1;24(3):20-3.

Human isolates of Salmonella Typhimurium DT104 in Ontario.

[Article in English, French]

Harnett N, Wan J, Brunins V, Borczyk A, Khakhria R, Johnson W.

Enteric Reference Laboratory, National Laboratory for Bacteriology and Enteric Pathogens, Ottawa, ON.

PMID: 9553281 [PubMed - indexed for MEDLINE]